European Science Foundation
Standing Committee for Life, Earth and Environmental Sciences (LESC)

ESF LESC EXPLORATORY WORKSHOP

Influence of Phytoplankton on Herbivore Reproductive Success – Impact of Infochemicals and Food Quality?

Scientific Report

Roscoff, France, 29 - 31 March 2006

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Executive Summary

28 participants from 8 European and 3 North and South American countries met from 29th to 31st of March 2006, at the Roscoff Marine Station (France), to discuss central achievements and future perspectives of work on the influence of phytoplankton on herbivore reproductive success with special focus on the impact of infochemicals and food quality.

Since the late 1950s, it has been assumed that nutrient limitation, competition, abiotic factors and predation determine demographic fluctuations of plankton species over the year. Till now most plankton models are based on this presumption. Thus it was assumed that food quantity and food quality are generally considered as key limiting factors for aquatic herbivores. Indeed, the evidence that food depletion in certain nutrients or other essential components limits the population of herbivores is documented in numerous cases. These descriptions of chemical interactions in plankton were mainly focused on few marker metabolic markers of food quality, such as polyunsaturated fatty acids, that are assumed to be limiting the herbivore success. The action of phytoplankton toxins arising mainly during mass occurrences of dinoflagellates or cyanobacteria (so called algal blooms) has also been addressed with strongly controversial results. Only recently, evidence accumulated that species interactions during the average situation in the plankton are also regulated by a “watery arms race” mediated by chemical or mechanical defenses. Because the majority of the involved infochemicals are either very dilute in the open water or only released during direct contact of the protagonists, most of the active principles are still awaiting discovery. Few examples of the direct action of chemical defense include the influence of diatom-derived polyunsaturated aldehydes on copepod embryo hatching and development and the role of dimethylsulfide as feeding deterrent.

The participants agreed that the understanding of the role of infochemicals in the plankton is a key for an in-depth insight into the interactions between primary producers and herbivores. It is remarkable that scientists with diverse expertise from modelers over geneticists to ecologists and chemists agreed that the action of infochemicals could explain several open unexplored problems in plankton research. Therefore, innovative approaches are needed that allow studying chemical fluctuations in field populations as well as chemical defense of single species and communities. Because the concept introducing infochemicals as a new regulating aspect challenges the established view of plankton ecology it is currently under controversial discussion. Existing studies on the role of infochemicals are often criticized to focus on one or only few parameters in laboratory experiments thus not reflecting the complex field situation in the plankton. In contrast, most traditional approaches to understand the processes that influence diversity are based on monitoring and modeling, two techniques that allow describing and to certain extend predicting the annual species succession in their natural environment. It is due to this methodological discrepancy that despite growing evidence, infochemicals are rarely discussed within the general framework of ecologically intact plankton. Proven effects are often considered rather as exceptions in an environment which is otherwise exclusively shaped by abiotic factors, food quality and quantity.

During this workshop these problems, all arising within or in-between the different disciplines were discussed. The participants were first providing an overview about the state of the art of their research and then pooled their expertise in working groups or plenary discussions to outline future research possibilities in the field.

Scientific content of the event

The first day of the meeting was devoted to a general definition of the state of research in this field. In four plenary lectures the participants were updated on the recent progresses in the relevant fields that should be discussed in this workshop:

- Impact of chemical communication on aquatic communities, by Ellen van Donk
- Molecular approaches to the study of genetic and ecological adaptation to the marine phytoplankton, by Linda Medlin
- Identification and evaluation of chemical signals in the sea, by Thomas Wichard
- Modeling planktonic predator prey interactions, by Kevin J. Flynn.

These plenary lectures were followed by 5 minute presentations of all participants outlining their most recent work and future concepts. The discussed issues included aspects of infochemical identification, biosynthesis and direct mode of action. In another focus, phytoplankton allelopathic interactions, such as reduced growth or induced lysis of conspecific and competing phytoplankton and protistan grazers were discussed. Central aspects of the influence of food quality and secondary metabolites on reproduction and growth of zooplankton were introduced. Modelers presented current approaches to incorporate influence of chemical defense for prediction of dynamics and energy flow in planktonic systems. Geneticists introduced approaches to apply established tools such as microarray and real time PCR in problems associated with the perception and effects of infochemicals in these systems. Interesting aspects arose from the interaction of researchers from the freshwater and marine fields.

After generating a common consensus on the state of the art of research in this field the participants split up in three working groups to define emerging new questions to fully address the complex effect of infochemicals and food quality parameters on plankton species-species interactions and plankton communities. Working group results were then discussed and structured in a plenary session. Emerging topics of interest are briefly summarized in the following paragraphs.

A general problem, which can be considered to be still open, is the question of whether and how toxin and "effector[1]" producing phytoplankton species benefit directly from a defense effect? A follow up question is whether these metabolites structure phytoplankton and plankton communities in general? Even if these seem to be trivial questions this aspect is hitherto not fully understood. Whereas it might be obvious that a unicellular organism can benefit from the production of toxins that prevent it from being predated, the action of metabolites that do not act directly is not so evident. In plankton systems even defensive metabolites that are not produced constitutively but only upon cell damage as it would occur during feeding by zooplankton are observed. In addition, defensive metabolites might not directly affect feeding activity but rather impair the reproductive success of the consumers. This type of delayed defensive mechanisms will have to be challenged in models as well as in field and enclosed experiments. In these systems cost issues are poorly addressed, including the metabolic costs of production and maintenance of these compounds, the ecological costs of inappropriate defense and the related implications for the evolution of chemical defense in plankton. To answer these questions we have to learn more about the biosynthesis of defense metabolites and the regulation of their production and release.

Methods and concepts are required to address the impact of allelopathy and whether defense metabolites can support bloom formation of selected species. On the other hand non-bloom forming species have yet to be tested for the presence and action of these types of metabolites.

It is till now not clear if a high genetic diversity of bloom forming species is also reflected in a phenotypic plasticity with respect to chemical defenses. If this is the case, the question would have to be addressed as to whether the beneficial effects are restricted to the single individual or to the entire blooming population. This would have major implications for explaining evolutionary aspects of defensive mechanisms. This question requires the establishment of links between traditional taxonomist and modern genetic methods, as well as the implication of analytical chemists investigating selected species and strains in laboratory and field experiments.

The cellular targets of chemical defense metabolites have to be investigated to better understand the observable effects in the individuals. We can learn a lot by studying those organisms that are not affected by defensive compounds since specific detoxification mechanisms can be expected. Especially, if we can identify species and clonal variability in susceptibility towards defenses comparable approaches facilitating this task will be enabled.

Studies on plankton chemical defense have mostly been limited to single metabolite studies, however this gives a rather incomplete picture since synergistic effects with other metabolites as well as overlaying effects of variable food quality and abiotic conditions have to be considered.

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[1] In the following we will use the term effector to describe secondary metabolites that influence herbivore success without necessarily being toxic.

Especially in limnological research it has been shown that chemical signals can induce morphologic defense and assist prey finding behavior for camouflage and protection. Still, the nature of these signals is in most cases not known. Similar processes in the marine environment are even poorer understood, even if they have the potential to significantly influence energy flow in-between trophic levels.

The workshop participants have identified a deficit in several methods and disciplines that would have to be overcome to solve efficiently key problems faced in plankton chemical defense and chemical communication. There is a need for a more efficient identification of relevant infochemicals, a problem that challenges chemists as well as ecologists. The implication of different modes of action of infochemicals in models is still poorly resolved. Genetic microarray and real-time PCR methods are still poorly adapted to the questions in plankton chemical defense. We still poorly exploit the powerful techniques of peptidomic, proteomic, transcriptomic and metabolomic to treat single species as well as the entity of a phytoplankton or zooplankton community. Recently transgenic phytoplankters became available, but till now these were not used to address key questions on the action of defensive metabolites. A major challenge for plankton ecology will be to overcome the mismatch between laboratory, mesocosm and field studies.

Assessment of results / future directions

Facing these central questions that challenge scientists from different disciplines working groups discussed further actions to facilitate a joint and synergistic research effort. The participants agreed that single projects relying on national funding resources would not allow synchronizing research in a field that essentially relies on multidisciplinarity. The group agreed to apply for an ESF EUROCORES program since this would enable scientific exchange and joint programs on plankton defense addressing problems that cannot be solved in individual efforts focusing on single or few parameters. To further support the development of a joint research program more specific topics were discussed in three workgroups.

Topic 1- How do we integrate freshwater and marine sciences?

This group identified the lack of direct comparative studies on the effect of phytoplankton food quality, infochemicals, chemical defenses and effects on community composition, as well as ecosystem structure and functioning. Despite several similarities between the two environments, interactions between marine and freshwater research are scarce. It would be thus interesting to perform a comprehensive meta-analysis of available data on chemical defense in the plankton communities in both aquatic ecosystems.

The design of two comparable enclosure experiments was discussed, that would focus on the collection of ecological relevant parameters for the direct comparison of the systems but also on the recording of all required data for a comprehensive accompanying modeling effort (see Topic 2 for further details). Comparative studies on the effect of aldehyde producing and non-producing phytoplankton species might be envisioned, but resistance of *Daphnia* sp., one of the most commonly used model herbivore in freshwater would have to be taken into account.

Comparative studies on the impact of kairomones could also result in a highly synergistic effect. Thus it is reported in both systems that herbivores react with a vertical migration behavior to the presence of their predators. The recognition of the predators is mediated by kairomones with hitherto unknown structures. Methodological as well as chemical parallel investigations would be really interesting for both fields. The joint investigation of kairomone induced colony formation of phytoplankton species in the presence of herbivores could also be envisaged.

Interesting aspects would also include a general comparison of the taxonomic diversity of algal blooms in both environments and the potential impact of diversity on the evolution of defensive mechanisms.

Topic 2- What are the required parameterizations and sufficient data sets to drive advances in plankton modeling?

Ideally, controlled experiments would be performed. For the investigation of the impact of chemical defense, these would include investigations of single prey, multiple prey, as well as single and multiple preys with predator(s). Both defended and undefended phytoplankton species would have to be compared. The experiments would be conducted in similar culture vessels under different nutritional conditions.

The data to be collected are of two types. Type 1- Mass balance data and major rate processes (e.g. abiotic parameters, nutrients, carbon/nitrogen/phosphor, chlorophyll, biomass, cell counts, growth rates of each component, detailed chemical analysis for nutritional factors, dietary factors and toxin contents, etc.). Type 2- Phenomenological data to enable the understanding of the underlying biology and ecology (they may or may not add information on rate processes or aid in modeling, but if we don't measure them at the same time then, we don't know what we may be missing).

Topic 3- Could we envisage a joint European mesocosm experiment collecting a comprehensive data set for in-depth understanding of selected systems?

The processes in the plankton are highly complex and cannot be observed in single parameter studies. Moreover high seasonal variations, annual fluctuations and regional differences make the combined data from different studies difficult to use, if not impossible to compare. To get a comprehensive picture, we would need to pool the expertise of scientists from different disciplines that work at one time on one selected system. The University of Bergen could offer a mesocosm platform, where 30-45 scientists could run a joint comparative experiment on the impact of chemical defense on plankton communities. An extended interdisciplinary research cruise might be envisioned as well, even if it would involve a major organisational and financial effort. Comparative approaches can be envisaged for freshwater research, where mesocosm experiments are already established as well. Special emphasis should be placed on a comparable set-up to reveal major similarities and differences between different lakes and the ocean.

As a synopsis of the above mentioned points the following types of parameters or measurements should be made to examine zooplankton feeding on phytoplankton that produces biologically active metabolites, such as polyunsaturated aldehydes, etc. The list is generic in that it is intended to be of use in both marine and freshwater systems, for whatever types of zooplankters are being examined.

Among key core parameters, envisaged for system comparisons, one should measure:

Hydrography: light, turbulence, temperature, salinity, nutrients (nitrate, phosphate, silicate, ammonium, urea, dissolved organic nitrogen, chlorophyll).

Phytoplankton/microzooplankton: quantitative species counts of organisms, epifluorescence microscopy (heterotrophic, autotrophic protists, bacteria), flow cytometry (live and preserved), flowcam. Microzooplankton grazing rates using e.g. fluorescently labelled algae, targetting specific species that contain chemicals of interest. Cultures should be established from isolated cells (c.a occurring during blooms in the field) to examine clonal variability.

Zooplankton: net tows or pumped samples (50-500 µm mesh) for quantitative community composition (preserved samples); samples for experimental studies on dominant taxa and/or those of interest to measure (grazing on phytoplankton/microzooplankton, egg production, egg hatching, larval development and mortality).

Chemical analyses: Eicosapentaenoic acid, docosahexaenoic acid, total fatty acids, steroids, vitamins, proteins, amino acids, sugars, polyunsaturated aldehydes, dimethylsulfide, phytoplankton toxins of interest, such as paralytic shellfish toxins, domoic acid, etc. A rather general metabolic profiling approach might be envisioned as well.

Genetics: for phytoplankton, ITS, microarrays and real-time PCR; for zooplankton, PCR/RNA for maternal zooplankter and her offspring, and DNA-based feeding rates (probes of taxa in the food and the gut of grazers), to determine the genetic diversity of predators and their preys.

Cellular Physiology: Herbivore responses (growth, reproduction, health fitness) to chemicals should be considered at the cellular level, such as cell degradations in guts and gonads, by using fluorescent probes, antibody labeling, ect. This could be done using some of the samples already collected (above).

Conclusion

This ESF exploratory workshop provided a fruitful platform for European scientists of different disciplines to define open questions in the field of plankton infochemical and food quality research. It also allowed the participants to pool their expertise and to use this synergy to outline future research perspectives that could allow a joint interdisciplinary effort for the investigation of highly complex plankton communities. Besides the direct initiation of collaborative research during this meeting, this workshop will also result in the preparation of an "*opinion paper*" defining the state of the art, open questions in this field and new perspectives (submitted as "Horizon" to the Journal of Plankton Research) and in the design of a joint research initiative for the EUROCORES program.

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FINAL PROGRAMME

Tuesday 28 March 2006

Evening	*Arrival of the participants at the Station Biologique de Roscoff, France*
19:30-21:00	*Open Buffet* (Hôtel de France, dining room)

Wednesday 29 March 2006

08:00	*Breakfast*
09:00- **ROOM n°3-**	**Welcome and introduction** (**Serge Poulet**, **Georg Pohnert**)
09:20	**Introduction of the participants** (three slides, maximum 4 minutes per participant, including 15 min coffee break) *Coffee break*
11:45	**Plenary introduction**: Impact of chemical communication on aquatic communities (**Ellen van Donk**, Netherlands)
12:30	*Lunch*
14:00- **ROOM n°3-**	**Plenary talk 1**: Molecular approaches to the study of genetic and ecological adaptation to the marine phytoplankton (**Linda Medlin**, Germany)
14:45	**Plenary talk 2**: Identification and evaluation of chemical signals in the Sea (**Thomas Wichard**, Germany) *Coffee break*
16:00	**Plenary talk 3**: Modelling planktonic predator-prey interactions (**Kevin J. Flynn**, England)
16:45	**Plenary discussion about first ideas for future approaches, definition of a maximum of 3 work-groups**
19:30	*Dinner Banquet* (Hôtel de France, dining room)

Thursday 30 March 2006

08:00 *Breakfast*

09:00- **ROOMs n°2,3,4-** Workgroups 1,2, 3 discussion ending with the preparation of a short statement

Coffee break

11:30- **ROOM n°3-** **Plenary discussion with statements of subgroups and definition of new goals**

12:30 *Lunch*

14-16-**Rooms n°2,3,4**- Workgroups n° 1,2,3 organization

16:30 *Departure to outing "The Ile de Baz"*

19:15 Depart from Ile de Baz

19:40 *Dinner*

21:00- **ROOMs n°2,3,4-** Workgroups 1,2,3 night session *(open end)*

Friday 31 March 2006

08:00 *Breakfast*

09:00- **ROOM n°3**- **Plenary session with report from subgroups, wrap-up discussion, European perspectives**

Coffee break

13:00 *Lunch*

Afternoon- **ROOM n°4**- *Departure of those participants not involved in the preparation of a final meeting report. Meeting of the steering committee for the preparation of a workshop documentation, to be submitted to a scientific journal such as Marine Ecology Progress Series.*

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Statistical information on participants

Origin of participants:

Germany (5)

France (5)

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Italy (4)

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Norway (2)

Denmark (1)

Switzerland (1)

Canada (1)

USA (1)

Chile (1)

Of these participants were 9 Female and 19 male, 4 were Junior scientists.